APOSPORIC PARTHENOGENESIS IN A TRIPLOID APPLE, MALUS HUPEHENSIS

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With plates 179–181

A NUMBER of years ago Dr. Karl Sax, in his attempt to make intergeneric crosses in Pomoideae, found that whenever *Malus hupehensis* was used as the female parent, seeds were always formed. Preliminary experiments were made to discover the basis of this peculiarity (Sax, 1931) and it was found that this species behaved as a typical parthenogenetic plant. I was designated to investigate the problem and the present report is a cytological analysis of this phenomenon.

Malus hupehensis (Pamp.) Rehd. (*M. theifera* Rehd.) (Rehder, 1933) is known to be a pure species, distributed widely in mountain regions of China extending south into Assam. It was introduced into this country in 1900. All plants of this species growing in the Arnold Arboretum, some of which have been propagated from seeds here and some brought in from outside sources, fruit heavily; the fruits containing the usual number of seeds. All the seeds appear to be deformed in outline in comparison with those of other species.

MATERIAL AND METHODS

In order to have a sounder basis for our conclusions concerning parthenogenetic behavior in *M. hupehensis* it was thought advisable to make parallel observations on another species, the embryonic development of which was normal. *Malus arnoldiana* (Rehd.) Sarg. (*M. baccata* × *floribunda*), a hybrid, chosen as a control plant, produces an abundance of fruit, and normal seeds in contrast to *M. hupehensis*.

In the summer of 1931 a preliminary examination was made of the chromosome number from root-tips of a *M. hupehensis* seedling and it was found to be a triploid form. The analysis of the ovules was made beginning the first week of May and continuing through the summer during the years 1932, 1933 and 1934. The study of this problem during the first two years was exploratory in character and it was in 1934 that a more complete analysis of the ovules of both *M. arnoldiana* and *M. hupehensis* was made.

Specimens at different stages of ovular development were fixed separately. The fixing of material was begun during the first week of May

when the buds began to turn pink and the development of the female meiotic phase was initiated. This phase is completed just before shedding of petals when the egg apparatus in the embryo sac (ES) is formed and fertilization has taken place (normally). From the pink stage to the time of shedding of petals six separate collections were made. Two collections were made between shedding of petals and remaining parts of perianth. After that the collections were continued at 5 to 7 day intervals in order to follow up the sequence of embryo development.

In fixing material for early stages, the fleshy part of the ovary is trimmed off with a sharp knife in order that the fixative may penetrate the ovules quickly. For later stages young seeds are removed from the fruit and are fixed directly. For quick penetration of fixative in older seeds the seed coat may be slightly slashed. For more mature seeds the whole testa may be removed.

The first year Flemming's solution was used as fixative but was found unsuitable for this material. Navashin's solution was found preferable for the meiotic and earlier stages of embryo sac formation. However, being doubtful of the effect of the latter fixative on later stages, Lewitzky's solution (5% formalin and 0.5% chromic acid) was used for those, since I had found earlier (Dermen, 1933) that it facilitates at least the staining of nucleoli with either crystal violet-iodine or haematoxylin. In this way I hoped to have at least the nucleoli to go by as landmarks, so to speak, in determining the nuclear number and other features in the development of embryo sacs. The results eventually showed this assumption to be justified. To prevent severe distortion of embryo sacs in changing fixed material into alcohol for paraffin sectioning, the material was allowed to remain in the fixative for twelve hours or more, washed in water and run up in alcohol gradually beginning with 5%. The results later showed that there was some shrinkage of the embryo sac and endosperm layer away from the nucellar tissue, though not enough to make observations confused.

Paraffin sections of early stages to the beginning of ES formation were cut 20 μ thick and from ES formation on up 35–40 μ, thick enough to obtain whole embryo sacs and young embryos, in order that confusion might be avoided in studying the origin and sequence of development.

Meiotic stages were stained with crystal violet-iodine, and ES and later stages with haematoxylin. Root-tips were fixed in Lewitzky's solution and stained with crystal violet-iodine; while for meiosis in the anther aceto-carmine technique was used.

Due to technical difficulties in illustrating various phases, it was necessary to use different scales. The scale used is indicated under each drawing.

DESCRIPTION

The features illustrated which were considered important in the analysis of parthenogenetic behavior in *M. hupehensis* are presented in 38 figures in three plates. The drawings are made in diagrammatic form to bring out the points pertinent to this study and to give the most essential details of origin and development of both embryo sac and embryo. The comparative illustrations of the control plant, *M. arnoldiana*, and *M. hupehensis* run from Figs. 18 and 19 to Figs. 34 and 35 inclusive. Other figures are from *M. hupehensis* alone.

The somatic chromosome number in *M. hupehensis*, studied from root-tips, was 3n = 51 (Fig. 1), a triploid number, the diploid being 2n = 34. The next figure (Fig. 2) represents the chromosome set from a tetraploid seedling of *M. hupehensis* with 4n = 68, the discussion of which will be found elsewhere in this paper. The size of these chromosomes is about one micron in length.

The meiotic development in anthers took place about 10 days earlier than the corresponding phase in the ovules. In the anthers the homologous chromosomes form trivalents and bivalents and a few remain univalents (Sax 1932). Figure 3 is II M from anthers with 25± chromosomes at each plate. No mature pollen grains are found in the anthers. Some cells begin to degenerate at I M and others at later stages, and most degenerate after they have reached the tetrad stage when the individual microspores fail to break apart although they are fully differentiated and each has its own wall.

A similar process of degeneration was found in the ovules. Here the chromosomes either remain univalents at I M or pair variously and divide at random. Figure 4 (a and b) represents I M in an egg mother cell (EMC). The chromosomes here are in univalent form. In the two sections, a and b, the total is exacly 51. It cannot be stated with certainty when, if ever, in univalent form there was any division in the EMC. However, in a few instances observed where the chromosomes were unpaired the cell was in a stage of degeneration. Such a cell is shown in Fig. 11. An anaphase stage is given in Fig. 5 (a and b). The b group of chromosomes from the lower pole was in a following serial section. Here also the total number of chromosomes could be accurately counted.

Altogether in *M. hupehensis* over two hundred ovules in the early stages of meiosis were examined and only rarely were there found ovules lacking a meiotic cell, either in prophase, in division stages, or in a degenerative process. There were found some older ovules which lacked embryo sacs. One young ovule was found with two egg mother cells.

There were observed many ovules divided into two nucellar regions, a twin ovule, each with a simple or a compound ES, enclosed together within a common integument.

An EMC is shown in Fig. 6 at pachytene stage. Usually around a meiotic cell there are one or more somatic cells each with a nucleolus as large or larger than that in the meiotic nucleus, which, as will be pointed out later, may develop into embryo sacs. Very rarely there were found ovules with two egg mother cells. Such a case is shown in Fig. 7. Around these meiotic cells are found a number of large nucleolated somatic cells. Figure 9 is a small scale drawing of the same cell to indicate the distance of a meiotic cell from the upper end of the nucellus. Here the EMC is 6 cell layers deep, while in other ovules the number of layers may vary. In *Lilium regale* (original observation) this layer was always one layer deep from an early stage to the time of fertilization (later stages were not observed). Figure 10 illustrates a typical young ovule with two layers of integumental tissue as it appears in longitudinal section. The cell in it is the same as in Figs. 8 and 9. The tissue around this cell is the nucellus. This figure shows the approximate location of the EMC in the ovule taken as a whole.

As was mentioned above the EMC may either degenerate at I M or later as in the anther. A degenerating cell at I M is shown in Fig. 11. Here the cell was found compressed from the sides and the chromosome stain was blurry showing these bodies more or less clumped together. As a whole its degenerated feature could be easily distinguished when compared with other cells at the same stage. As well as could be made out the chromosomes in this cell were not paired, which fact, I believe, may be correlated with degeneration of the cells at this stage.

Figures 12 and 13 show a parallelism in division with EMC and in development of one of the resulting cells into, supposedly, an ES. Normally an EMC should divide twice and from this division four cells should result. One of these, which is the furthest from the micropyle should be the megaspore destined to become an ES. However, as will be shown below, this expectation is not fulfilled in *M. hupehensis*. In Fig. 12 we find three cells. The one nearest to the micropyle has degenerated. This cell corresponds to one in Fig. 13 which was in metaphase and in a stage of degeneration. In this latter figure the second division was found to be irregular; some of the chromosomes at opposite poles were at a distance from the main chromosome groups. If this cell divided it would result in two unequal sized cells, because these two groups of chromosomes are not found at equal distance from the ends of the cell. This is what is found in Fig. 12. Here one cell is degenerating.

One of the sister cells from the second division is small, the other large, and in this large cell there is a small chromosome mass outside the main nucleus, indicating that this cell is not an ordinary somatic cell but the product of an irregular second division of an EMC.

Direct as well as indirect evidence shows that meiotic cells at some stage of their development degenerate while somatic cells in their immediate vicinity usually grow in size and develop into embryo sacs. Figure 14 illustrates this point clearly. Here is shown a rather large somatic cell with a large nucleolus, above which there is a meiotic cell in a stage of degeneration. In Fig. 15 is shown such a cell that has grown in size. The nucleus has divided and the cell is in a two-nucleate stage and beginning to develop into an ES. At the base of this are shown four cells in a row that have annular xylem thickenings, an unexpected manifestation in an ovule, this type of cell being characteristic of vegetative tissues. Similar cells were found at later stages of ES development and in one case in a complex ES. Serial sections from either side of this were scrutinized and no trace of degenerating meiotic cells was found in the neighborhood of this binucleate ES. Therefore, it may be that these four annulated cells represent the tetrad resulting from two meiotic divisions. Their degeneration was expressed by transformation into xylem elements. Such cases were found only in 1932 material.

In the majority of cases the embryo sacs were structurally complex. These were found to have resulted from the fusion of two or more embryo sacs. A simple case is shown in Fig. 16 where two embryo sacs were found growing simultaneously. In other cases the number of these young embryo sacs growing side by side may reach six or more. In this figure one is four-nucleate and the other below it two-nucleate. In Fig. 23 two adjacent sacs are shown in mature egg apparatus stages. Figure 17 represents the middle section of five serial sections in which a total of 30 nuclei could be counted which may have resulted from fusion of four embryo sacs, boundaries of which could not be made out clearly.

Illustrations from Fig. 18 to Fig. 35 inclusive are diagrammatic representation of comparative development of embryo sacs and embryos in *M. arnoldiana* and *M. hupehensis*. Figures 18 and 19, the former from *M. arnoldiana* and the latter from *M. hupehensis*, are from material fixed at the time of full bloom. Both represent the stage when the embryo sacs have completed the cycle of the development with three antipodal nuclei cut off, each having formed a separate small cell. The main portion of the ES now functions as a distinct body in which there is an egg cell with two synergid cells together forming the egg apparatus, below which the two polar nuclei have approached each other but com-

plete fusion is delayed, perhaps till joined with a sperm. A similar situation was observed in *Lilium regale* (original observation) where first the upper polar nucleus is joined with a sperm and these two jointly meet half way with the lower polar nucleus. By the time of fertilization the egg cell is larger than the synergids (Fig. 20, *M. arnoldiana*). In these drawings the upper limits of the egg and synergid cells are not shown because they were vague in my preparations. In Fig. 19 (*M. hupehensis*) a dotted nucleolus of one of the polar nuclei and a dotted synergid cell are superimposed on the main portion from an adjacent section in a single drawing. The outer broken line represents the inner limit of the nucellus. A more mature ES is shown in Fig. 20 of *M. arnoldiana* from material fixed at the time of calyx shedding (the last remnant of the perianth with dried up styles). Here only nucleoli are shown. At this stage the antipodal cells had disappeared and the sac had grown longer but not much wider. At this time of the ES development fertilization has usually taken place, but perhaps due to climatic conditions some cells remain unfertilized. In the ES shown in Fig. 21 (*M. arnoldiana*) there is a clear indication that fertilization has taken place, judging by the amount of nucleolus in the egg nucleus in comparison with the same in the synergids. In the other figures (Figs. 18–20, taking these individually) the size of the nucleoli in the egg and the synergids is remarkably similar. Fertilization in Fig. 21 is further indicated by the fact that there appears the beginning of endosperm nuclear division, due to fusion of the polar nuclei with a male gamete.

The embryo sacs shown in Figs. 22, 23 and 24, all of *M. hupehensis*, are from material fixed at the time of calyx shedding as in Figs. 20 and 21 of *M. arnoldiana*. These five figures are drawn to the same scale. In all the antipodals have disappeared. Judging from the size of the nucleoli of the cells in the egg apparati in the three figures (22, 23 and 24), as in Fig. 20 pointed out above, there is no indication of fertilization and these cells will remain unfertilized since the fruits at this time are quite advanced and the styles have long since dried up and dropped off with the remaining part of the perianth. In Fig. 22 we see the beginning of endosperm formation after, perhaps, polar nuclei have fused together and divided without being fertilized. The egg cell grows larger, its nucleus moves to the tip of the cell, the synergids eventually disappear and long after there sets a cell division at the tip of the egg cell. From now on the ES begins to grow lengthwise and extends through the nucellar tissue in a manner similar to pollen tube growth. The nucellar cells in the neighborhood of the sac begin to break down, undoubtedly due to some sort of enzymic action, offering no resistance

to the tubular growth of the ES. In Fig. 23 we find two embryo sacs side by side. The missing parts of these are given at the side of the twin sacs drawn from an adjacent section. Here a fused polar nucleus is shown under one of the cells of the egg apparatus, indicating that polar nuclei may fuse before the endosperm development is set. Figure 24 represents a single ES; one of the cells shown at the side is drawn from an adjacent section.

Tubular growth of the ES in *M. arnoldiana* is in proportion to the embryo growth. About a week after fertilization the number of cells in longitudinal section of the embryo is about six. At this time the tip of the ES is ½ to ⅔ the length of the nucellus. In *M. hupehensis*, when the ES is a derivative of only one somatic cell, the width of the sac is about the same as that of *M. arnoldiana*, but in the great majority of the ovules examined, the ES has grown disproportionately wider and deeper in the nucellus after reaching its basal limit, independently of the amount of endosperm as well as embryo growth which at this time may still be in the one cell stage. This is undoubtedly due to the compound nature of the ES, as shown for example in Figs. 17 and 23. Figure 25 represents a very young embryo (proembryo) of *M. arnoldiana* about two weeks after fertilization. At this time the number of cells in the proembryo in longitudinal section averages ten. The tip of the ES has barely reached the lower limit of the nucellus. Figure 26 shows diagrammatically this stage. The broken line in this figure indicates the extent and the limit of the ES while the dotted line at the base shows the basal tissue which is firm and not penetrated by the ES. In Fig. 27 is shown the beginning of proembryo formation in *M. hupehensis*, two weeks after shedding of petals.

The embryo development in *M. arnoldiana* is regular and rapid compared with that of *M. hupehensis*. About three weeks after fertilization the proembryo in *M. arnoldiana* differentiates into a suspensor and a true embryo (Fig. 28). In this figure the endosperm, composed of a uninucleate layer, is separated from the nucellar tissue due to shrinkage from fixation. In *M. hupehensis*, if any embryo development is initiated, the embryos are still in proembryonic form at a corresponding period. Such a stage is shown in Fig. 29. This drawing represents a compound ES in which two proembryos were found, one at the micropylar corner and the other in the free space, away from the nucellus, each undoubtedly belonging to a separate ES, the limits of which cannot be easily distinguished. A four weeks old embryo of *M. arnoldiana* is illustrated in Fig. 30 showing the suspensor, the true embryo and more extended growth of endosperm tissue, the broken line indicating its limit of growth.

The central region of the ES is still devoid of this tissue. In *M. hupehensis*, four weeks from the time of flower shedding, the proembryo is still undifferentiated. Such a stage is illustrated in Fig. 31. In this case a single proembryo was found in the middle of a complex ES. Eventually other embryos may develop in the same sac. This particular ES was chosen for illustration merely to indicate that in *M. hupehensis* embryos develop, as seen in prepared material, directly from the ES and never from any other tissue surrounding or near the ES; otherwise, more often, the embryos are located at or near the micropylar corner. Figure 32 represents a six weeks old embryo in *M. arnoldiana* when the cotyledons begin to differentiate. The broken line indicates the limit of a dense endosperm layer next to the nucellar tissue. This layer seems to separate the suspensor from the nucellus and extend between these. The embryo is imbedded in looser endosperm. Figure 33 shows the stage of embryo development in *M. hupehensis* six weeks from flower shedding. Here there is the beginning of differentiation of the proembryo into a suspensor and a true embryo with few endosperm nuclei in the sac. Figure 34, drawn to a smaller scale, shows a five weeks embryo from a wide view of the cotyledon in *M. arnoldiana*. The broken line shows the limit of the dense endosperm, the embryo being imbedded in loose endosperm. The outer two solid lines indicate the limit of the integumental tissue. The extent of embryo development in *M. hupehensis* at a corresponding period is shown in Fig. 35 drawn to the same scale used in figures preceding Fig. 34. Broken lines indicate the extent of endosperm growth which is shrunk away from the nucellus. The middle area is still devoid of such growth.

Often in *M. hupehensis* there were found twin ovules, each with its own ES, both enclosed in a common testa. In some the demarcation between the twins is quite distinct, in others vague. In Fig. 35 only the upper limits of such twin ovules are shown, where the demarcation, shown by a dotted line, could hardly be distinguished. At the left side of the suspensor in this figure there is a bulge which may indicate that at this point another true embryo may be initiated resulting in a "siamese twin." In more mature stages I have observed structurally complex embryos which may have been originated from such a process of development.

The above ends the description of comparative illustrations for *M. arnoldiana* and *M. hupehensis*. The following description is based on *M. hupehensis* material given in Figs. 36–38.

Figure 36 represents an embryo from material fixed two months after shedding of flowers, which is still in a premature stage; while in *M. arnoldiana* the embryos were fully developed and filled the whole

nucellar area. The sac in this particular ovule is divided into two portions indicated by a dotted line. The side which contains the embryo is filled with loose endosperm cells. The other side has only a narrow strip of endosperm shown in the drawing with broken lines. Examining the serial sections, at the upper corner of this portion there was found a very young embryo. As a whole the embryos at this late period of development did not vary much from the one shown in this figure. An enlarged drawing of this embryo is given in Fig. 37. The cells in the suspensor are drawn loosely since their actual limits could not be made out clearly. The round tip represents the beginning of development of the true embryo. A three months old embryo, from a narrow view of cotyledons, is given in Fig. 38 drawn to the same scale as Fig. 34. Between the cotyledons the epicotyle is beginning to be formed. In this drawing the testa which was removed from the seed for better penetration of fixative is not shown. At this period the embryo is ⅓ to ½ way down in the sac and has not yet replaced the endosperm as well as the nucellus.

DISCUSSION

The title of this paper is meant to suggest that embryogeny in *M. hupehensis* occurs by means of development of unfertilized egg cells and further, that embryo sacs in the ovules develop from other cells than megaspores or megaspore mother cells (egg mother cells). The terms *apospory* and *parthenogenesis*, as here applied are meant in the following sense: *Apospory* signifies the development of an embryo sac from a cell other than a megaspore or megaspore mother cell; *Parthenogenesis* signifies the development of an embryo from an egg cell without fertilization. The two processes are included in the term *Apomixis* which signifies the absence of sexual fusion in the reproductive process.

Almost without exception in the ovules of *M. hupehensis* there is one typical meiotic cell that can be recognized by its synezetic chromosomal appearance in early stages. Rarely a meiotic cell is not recognized in this or in an advanced stage. Both in the ovules and in the anthers there is an attempt at reduction in meiotic cells. In the anther the great majority of cells reach the tetrad stage with a wall around each of the resulting cells. The component microspores of the tetrads, however, do not break away and their development ceases at this stage and eventually the tetrads degenerate. Some cells do not even go through reduction but stop at metaphase. A comparable situation exists in the ovules where, if meiotic division is at all successful, one of the resulting megaspores may tend to grow somewhat, while others have ceased to grow or have already disintegrated. However, the author's impression

is that probably never in this species is a megaspore developed into a functional ES. All the evidence available tends to show that the embryo sacs are of aposporic origin.

As was described in the text, in the ovule, in the immediate vicinity of a meiotic cell, there is usually more than one somatic cell with a large nucleolated nucleus which in almost all ovules develops into a typical eight-nucleate embryo sac which sooner or later forms the egg apparatus. A few ovules were found which were devoid of embryo sacs. The ovules themselves continued to grow. In most cases the embryo sacs were complex in appearance and were found to have resulted from the fusion of two or more aposporic embryo sacs. In such embryo sacs more than one egg apparatus is formed, from which the appropriate number of embryos and appropriate number of endosperm layers could be formed. No indication whatever was found of development of embryo sacs and embryos from any other tissue than described in the text. The exact origin of these parts could be fully demonstrated since a very large number of ovules at all significant stages was examined. Furthermore, these stages were carefully checked by using as control a diploid form of apple in parallel observations and there were found no exceptions to the rule indicated above.

Kobel (1931) refers to "diploid parthenogenesis" in an apple variety, "Transparent von Croncels." No reference was found in the literature on apples giving any account of such a phenomenon. Liljefors (1934) has shown apospory to be present in two tetraploid species of *Sorbus*. Rosenberg (1908) describes a similar phenomenon in *Hieracium*. He finds that the greater number of the embryo sacs in the ovules in *Hieracium* are of aposporic origin, and that "Simultaneously with the development of this vegetative cell into an embryo sac, the embryo sac mother cell is, as usual, divided into tetrads with the reduced number of chromosomes, and a normal embryo sac commences to develop. This however, most usually, is sooner or later destroyed by the encroaching aposporic embryo sac. Still in some cases two embryo sacs could be fully developed: one probably typical and the other aposporic. In the same head some ovules have a typical and others an aposporic embryo sac." As in *Hieracium*, in *M. hupehensis* there is a typical meiotic cell present, but apart from that only aposporic cells develop into functional embyro sacs. Rosenberg has made no mention of this point.

To show definitely whether reduced cells play any role in embryo development, a large number of seeds was planted from which a very small number of seedlings was obtained. Of a total of 39 seedlings, two were tetraploids, one had some roots showing the triploid number and

some hexaploid (this plant was destroyed after transplanting; if grown it might have developed into a chimeral plant since such an effect was already noticeable in the seedling), and 36 were triploids, one of which showed a more vigorous growth than others. Tetraploid as well as a few other sister triploid seedlings are now saved for future study. To be positive whether any reductional megaspore actually entered into the formation of a mature ES, the chromosome number in the ES nuclei or anywhere else during embryo development should be studied at the time when some nuclei or cells may be found dividing. Such an examination was not made since in this respect the fixation of my material was not as successful as could be desired. However, based on the observed facts recorded from studying numerous ovules at early and later stages of ES development and the fact that no aneuploid chromosome number could be found in the seedlings examined, we may be justified in concluding that although there is usually a meiotic cell present in the ovules, the embryo sacs, however, develop only from somatic cells in the immediate vicinity of the meiotic cell, at which time meiotic cells degenerate. When the rate of embryo development in *M. arnoldiana* is compared with that of *M. hupehensis*, as was pointed out in the text, the embryo development in the latter was very slow without a single exception. Very few seeds at maturity in *M. hupehensis* were at all fully filled; for the most part they were empty or contained diminutive embryos indicating their somatic origin.

It is a noteworthy phenomenon that in *M. hupehensis*, although an egg cell with a somatic number of chromosomes can develop into an embryo parthenogenetically to insure the stabilization as well as the propagation of the species, this same egg cell is at times receptive to fertilization with a haploid male gamete derived from a diploid species. Considering it in more practical terms, this type of hybridization if successful, may be used as a method of generation of new species artificially, considering that this combination occurred normally with no application of artificial methods. It may be assumed that some of the tetraploid species found in nature have originated in this fashion.

Triploidy in itself is an indication that *M. hupehensis* is a derivative either from a similar species but of diploid form, or from a hybrid between two species and later, through one haploid gamete being fused with a diploid gamete, or from a cross between a tetraploid and diploid species. In any case, the polyploidy in this plant seems to have brought about a genetic unbalance by virtue of which normal sexuality was suppressed but not enough to suppress the inherent property of the ovule to produce a gametophyte, although of sporophytic origin, thus insuring

alternative process of generation fundamental in almost all the plant kingdom. A genetic unbalance due to triploidy seems to have brought about a vegetative tendency of which parthenogenesis is the result, which in itself may be considered as being a vegetative process. In extreme cases the same tendency is expressed in the differentiation of nucellar cells into conductive (xylem) elements (Fig. 15) which are characteristic only of vegetative tissues while nucellar cells serve as a food source for a gametophytic growth.

The same factor seems to be at play in the disintegration of sexual cells in the ovules where these cells begin to degenerate at times even before they have begun to divide. The destruction of these cells in *M. hupehensis* is not a physical process of encroaching of an aposporic ES over a normal one, as Rosenberg reports for *Hieracium,* as if there were a competitive growth between these embryo sacs, but a process likely physiological, since egg mother cells begin to degenerate before an aposporic ES has commenced to develop.

Usually simple hybridization between any two species is not sufficient to lead to parthenogenesis or apomixis, in general, and such a method of propagation seems to be commonly linked with genetic unbalance through polyploidy. The data presented by Gustafsson (1935) in Table 8 seem to substantiate the validity of such an assumption. He presents the data of ten genera all of which in diploid form, with the exception of *Potentilla,* are sexual. Apomixis appears to be prevalent whenever the genom is in odd numbers, 3x, 5x, . . ., 15x the basic number of chromosomes. On the other hand in polyploids with an even number there are both tendencies, apomictic as well as sexual, present.

The prevalence of apomixis in polyploids with odd chromosome numbers may be usually due to a cytogenetic unbalance brought about by random and unequal distribution of chromosomes so that in some species such megaspores may not develop into functional embryo sacs. The triploid apple species studied in this paper differs from ordinary triploids in the fact that sexual cells may degenerate even before a first division is accomplished and not as a result of random and unequal distribution of chromosomes. In this laboratory we analyzed the cytological situation in the Baldwin apple, a triploid form, and found an approximately normal distribution of chromosomes from germinating seeds (see page 106). Here neither the polyploidy nor unequal distribution of chromosomes had any apparent effect on the normal sexual processes.

If specific genes were responsible for the apomictic phenomenon, as Gustafsson seems to argue, then the same phenomenon should have been

equally common in diploid hybrids, which is not the case judging from the table he presents.

The present author's contention is that the parthenogenetic process is fundamentally a vegetative act on the part of the egg cell growing into an embryo without uniting or being stimulated by a sperm. As a whole, in plants, parthenogenesis seems to be linked with polyploidy which accelerates vegetative growth and decreases normal sexual development. Crane and Lawrence (1930) have remarked that in apple "triploids are invariably vigorous." In a hybrid of *Mahonia* × *Berberis*, two closely related genera in Berberidaceae, there is a complete suppression not only of sexuality but even of formation of sexual parts such as flowers. The whole plant is completely vegetative and vigorous in growth. This hybrid has the same number of chromosomes as its parents with 2n = 28 (Dermen 1931). Obviously here polyploidy is not the cause of this peculiarity but it may be due to extreme vigor brought about by combining the two species of closely related genera. We have made attempts to make some artificial hybridization between the above two genera; if successful, the study of the intergeneric plants as well as the full analysis of tetraploid forms derived in *M. hupehensis* mentioned above should throw a good deal of light on the attempts to determine some of the causes of parthenogenesis.

Taxonomically *M. hupehensis* must be considered as a very stable species since very rarely will it fail to breed true from seeds. Most diploid species are more or less cross fertile with other species within the genus and when grown together they will fail to breed true from seeds; hence their propagation must be insured by grafting or by other vegetative methods. *Malus hupehensis* in this respect cannot be considered as a typical species since it differs from the normal in that it may safely be propagated from seeds although grown side by side with other species. As we have pointed out the seeds here develop parthenogenetically and very seldom through fertilization, in which case we may obtain entirely new cytological forms, as was described in the text. It is safe to assume that *M. hupehensis* is of hybrid origin and is consequently an allopolyploid form cytologically, since we assume that mere autopolyploidy through fusion of a haploid and a diploid gamete in a pure species will not bring about a genetic unbalance which would eventually have resulted in parthenogenesis. Some species in Pomoideae that are found to breed true under similar conditions, from seeds, may be suspected of similar phenomena as those described for *M. hupehensis*. This analogy may be extended to other species of similar behavior in other families.

SUMMARY

Malus hupehensis (Pamp.) Rehd. is a triploid species with 3n = 51 chromosomes. Sexuality is completely suppressed through degeneration of sexual cells in both anthers and ovules; however, the propagation of the species is carried on by parthenogenetic method. Evidence of hybrid origin of this species is indicated.

In the ovules embryo sacs are probably entirely formed by apospory in which normal egg apparati are formed. It is the egg cells alone that develop into embryos, and embryos never are formed from any other source. Polyembryony in *M. hupehensis* is found to be linked with the development of complex embryo sacs.

Based on the fact that parthenogenesis is commonly associated with polyploidy, a suggestion is made that the expression of such a phenomenon in *M. hupehensis* may be due to a physiological change brought about by triploidy and that this factor accelerates the vegetative growth in the plant and decreases or entirely suppresses its normal sexual development.

LITERATURE CITED

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EXPLANATION OF PLATES 179–181

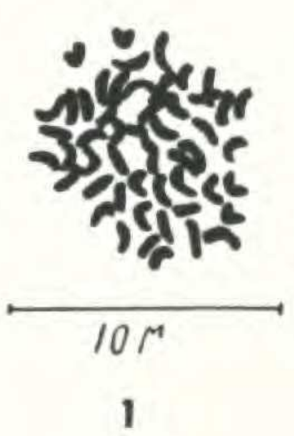
Figs. 1–17 *Malus hupehensis*

Fig. 1. Triploid number 3n = 51 chromosomes from root-tip sections.
Fig. 2. Tetraploid number 4n = 68 chromosomes from root-tip sections.
Fig. 3. II M from anther with 25± chromosomes at each pole. Aceto-carmine preparation. Material prepared between last week of April and first week of May, about ten days before buds turn pink.

Fig. 4. Egg mother cell (EMC) at I M with 51 univalent chromosomes from two sections a and b. Ovaries fixed from buds at pink stage, May 10, 1932.[1]
Fig. 5. EMC at I A in two sections. Pink stage, 5/10/32.
Fig. 6. EMC at pachytene. Pink stage, 5/9/32.
Fig. 7. Two EMCs at late pachytene. Pink stage, 5/10/32.
Fig. 8. EMC at I M. Pink stage, 5/9/32.
Fig. 9. Same as Fig. 8 showing cells in vicinity of the top of the nucellus.
Fig. 10. Outline drawing of an ovule showing the EMC in Fig. 9, the nucellus and the integuments.
Fig. 11. An EMC at I M degeneration. Pink stage, 5/10/32.
Fig. 12. Tetrad stage of megaspores. Upper one failed to divide and degenerated, the large one grown somewhat and its being of a meiotic derivative is indicated by a small nuclear mass outside the large nucleus. Petals falling stage, 5/10/32.
Fig. 13. Two daughter cells from an EMC division, one degenerating and the other at anaphase stage. Some of the chromosomes were off the plates, showing meiotic origin of these cells. Petals just opening stage, 5/10/32.
Fig. 14. A somatic cell with a large nucleolus growing into an ES aposporically, while the meiotic one above is degenerating. Petal just opening stage, 5/10/32.
Fig. 15. Two-nucleate aposporic ES and below it a tetrad of cells (of meiotic origin?) with xylem annular thickenings. Open bloom stage, 5/9/32.

[1]Pink stage buds usually at 5th of May in the Arnold Arboretum.

Fig. 16. Two aposporic embryo sacs, one two-nucleate and the other four-nucleate. Flowers just bloomed stage, 5/10/34.
Fig. 17. A middle section of a compound ES with some 30 nuclei in five serial sections. Flowers just bloomed stage, 5/10/34.
Fig. 18. *M. arnoldiana.* A complete ES with egg apparatus (egg cell and synergids), two polar nuclei and three antipodal cells, before fertilization. Before shedding of petals, 5/11/34.
Fig. 19. *M. hupehensis.* A complete ES as in Fig. 18. One polar nucleolus and one synergid cell, dotted, from an adjacent section superimposed in a single drawing. Before shedding of petals, 5/10/34.
Fig. 20. *M. arnoldiana.* An unfertilized ES. Antipodals have disappeared. Just about at shedding of calyx, 5/19/34.
Fig. 21. *M. arnoldiana.* A fertilized ES. Otherwise same as in Fig. 20.
Fig. 22. *M. hupehensis.* Endosperm development and tubular growth of ES. At shedding of calyx, 5/18/34.
Fig. 23. *M. hupehensis.* Twin ES. Parts drawn at the side from an adjacent section. Antipodals have disappeared. From same material as Fig. 22.
Fig. 24. *M. hupehensis.* A single ES. One of the cells of the egg apparatus drawn from an adjacent section. From same material as Fig. 22.
Fig. 25. *M. arnoldiana.* Proembryo stage with scant endosperm. Material fixed 5/31/34.
Fig. 26. *M. arnoldiana.* Longitudinal section of a whole young seed at the time of proembryo about 12 cells long. This shows the extent of tubular growth of ES. Material same as in Fig. 25.



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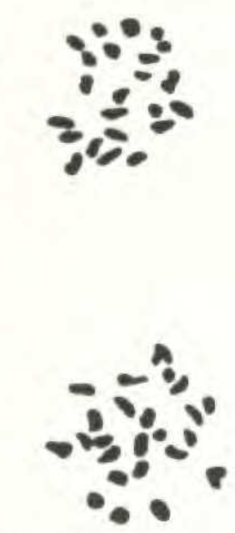

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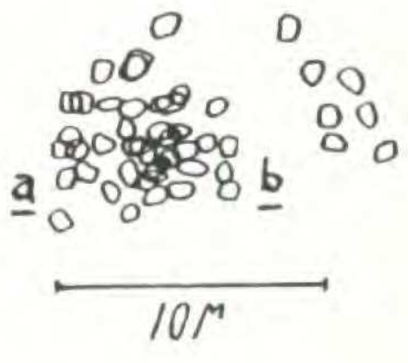

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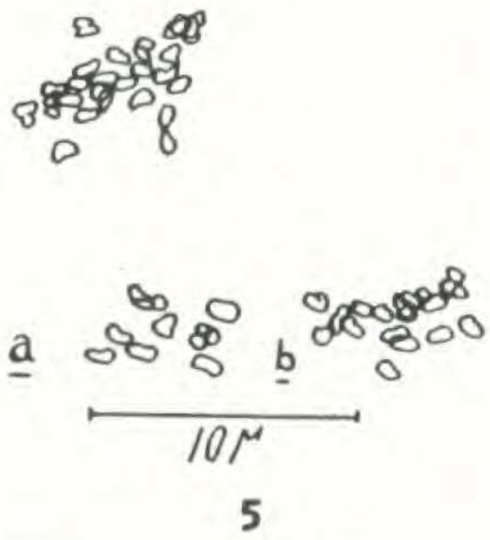

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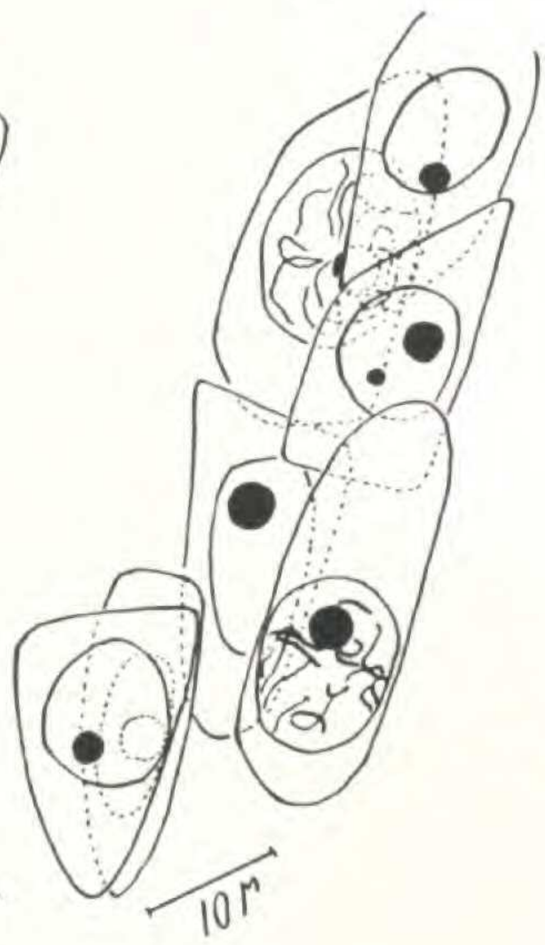

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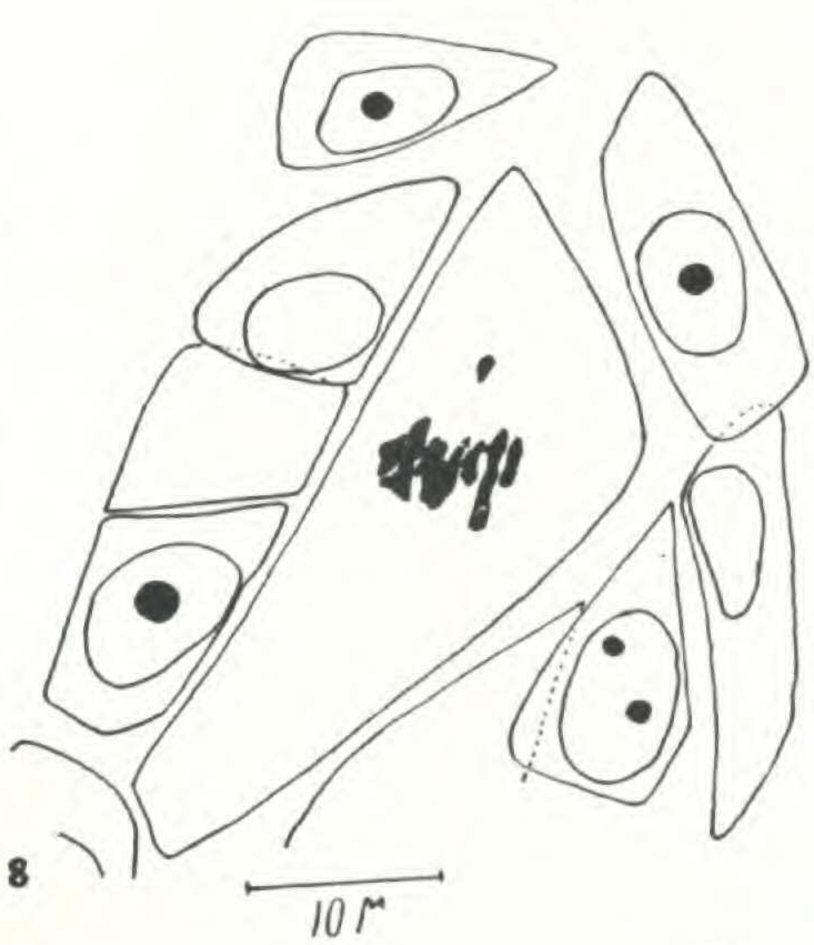

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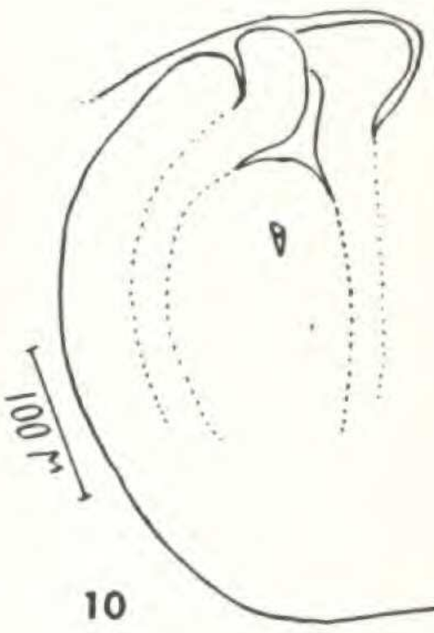

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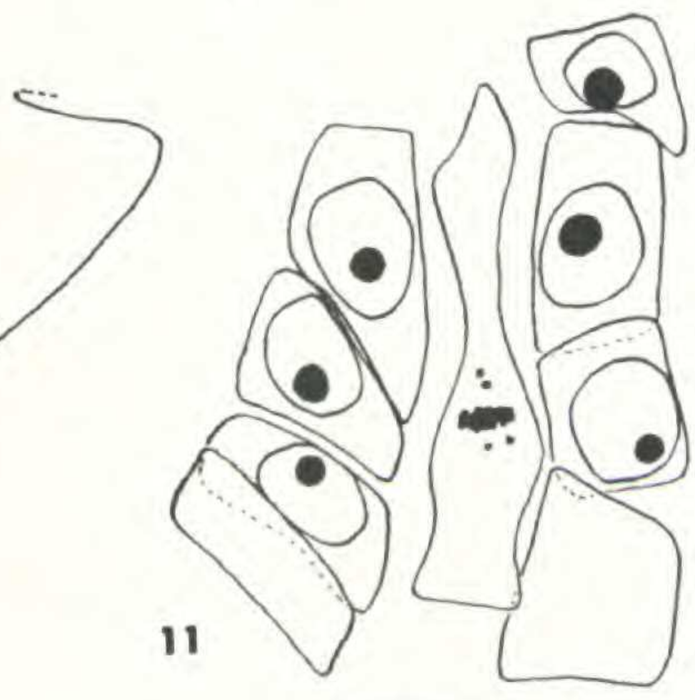

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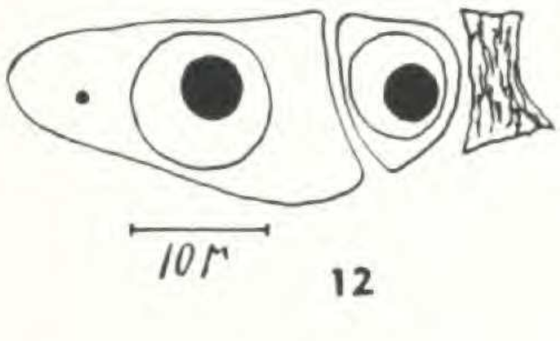

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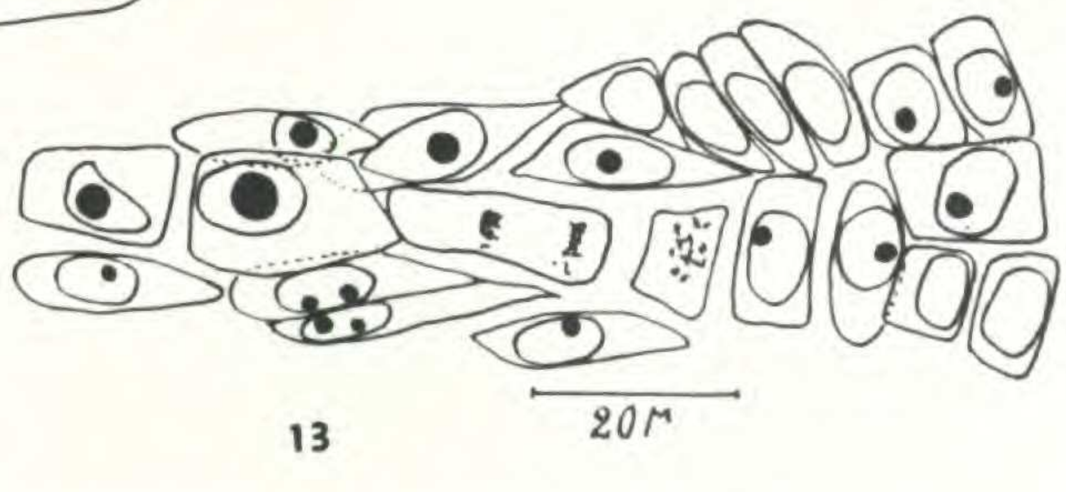

13

APOSPORIC PARTHENOGENESIS IN MALUS HUPEHENSIS